BIOS 318 : MICROBIOLOGY LABORATORY Spring 2022

Welcome to BIOS 318, Microbiology Laboratory

The 30,000 or so species of bacteria that have been identified by microbiologists[1] represent a very tiny fraction of the estimated number of bacterial species on Earth. Applying ecological scaling laws to data from over 35,000 surveys of microbial populations, researchers have estimated that from 100 billion to 1 trillion species of bacteria, archaea, and fungi live in our soil, in oceans, on animals and plants, etc.[2] This means that for every bacterial species successfully isolated from an environmental sample, as many as 10,000 species remain undetected. If the one trillion estimate is accurate, then if 100 Rice students *each* discover 100 new species every year (obviously not a realistic expectation), it will take 100 million years to discover all of them.

Our project

As a class we will attempt to isolate and identify species of *Streptomyces* bacteria, to support some very cool studies that are currently under way in the Shamoo lab, Department of BioSciences, Rice University.

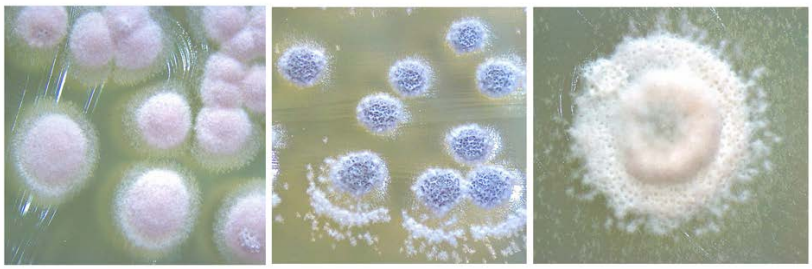

Fig. 1. (left to right) colonies of isolate T8-JL-3; colonies of isolate T5-ML-1, and a single colony of isolate T7-NS-2, all from the class of fall 2020. Photos by Lauren Eveland, BioSciences Dept.

It has been proposed that positive and negative interactions among bacterial strains define much of the ecology of soil. The Shamoo lab is studying the evolution of such interactions. *Streptomyces* are the source of the majority of antibiotics in use today, thus a possible side benefit of this research is the discovery of new antimicrobial substances. It's a long shot, but quite possible.[3]

Fig. 2. (left) Dilution streak plate of isolate T7-NS-2; (middle and right) streak plates testing for competitive interactions between isolates. Photos by Lauren Eveland, BioSciences Dept.

Each team of students will provide a soil sample from which to isolate *Streptomyces* cultures. After identifying potential *Streptomyces* colonies you will propagate isolates to obtain pure cultures and characterize them by phenotype and through 16S rRNA sequence analysis.

Objectives and learning outcomes

The main goal of this course, as must be true for any experiential course, is personal development. We want you to build on skills that you've already acquired and learn/apply new ones. Below we summarize our most important learning objectives and outcomes.

1. Learn to work as a productive member of a research team on a long term project
2. Be able to access and employ published reference materials, current primary literature, and other information sources in order to advance your understanding of the properties of bacteria and how to isolate and characterize them
3. Learn to integrate your previous and newly acquired experience into development of new techniques and strategies
4. Improve your powers of observation, such as the ability to use physical and metabolic differences to distinguish strains and species of bacteria and to draw information from microscopic observations
5. Learn to make the decisions that are necessary in order to conduct a systematic open-ended investigation
6. Learn to make your work accessible to yourself and others by documenting your activities and findings, by contributing to our laboratory's database, and by summarizing your work in a clearly written report
7. Learn to appreciate the value and potential of pure science, that is, the pursuit of knowledge for its own sake
8. Acquire proficiency in essential methodologies and use your newly acquired skills to advance our project
9. Develop the habit of practicing good laboratory "citizenship," including respect for people, the facility, and equipment.

Please see the Syllabus for course details, course timetable, and a list of assignments with due dates.

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[1] Dykhuizen, D. "Species numbers in Bacteria." Proc Calif Acad Sci. 2005 Jun 3; 56(6 Suppl 1): 62–71.

[2] Offord, Catherine. "Earth: Home to 1 Trillion Microbial Species." The Scientist (2016): http://www.the-scientist.com/?articles.view/articleNo/46016/title/Earth--Home-to-1-Trillion-Microbial-Species/

[3] https://phys.org/news/2018-12-bacteria-ancient-irish-soil-halts.html

BIOS 318 Spring 2022 Syllabus

Welcome again to our microbiology laboratory. Here you will find information about the course, instructors, policies, and a timetable of events. ***Details are subject to change at the instructor's discretion.***

COVID policies

*****As of Monday, Dec 27, 2021 we will go virtual for the first two weeks of classes; expect the schedule to be modified accordingly*****

We should have had this thing beat by the end of April last year, and then the fools (and there are many of them) ruined it for us. We hope that we can have as close to a "normal" semester as possible. Nevertheless, we still have to exercise COVID-related cautions. University policies apply to this lab, of course. If you feel ill, please stay home. All of us must be masked when in the laboratory (exception noted below) and masks must cover mouth and nose. When your instructors address the class during the course introduction and at other times at the beginning of class we will be unmasked and keep at least 10 feet from the nearest student. When we circulate among you we will remain masked for the duration.

Instructors and office hours

David R. Caprette, Ph.D.
Teaching Professor, BioSciences, caprette@rice.edu
Office: ABL 102B (Biology Building, first floor west).
Office hours outside of lab will be by appointment, arranged via email or requested during class.

Collin Thomas, Ph.D.
Lecturer, BioSciences, collin.thomas@rice.edu
Office: ABL 101B

Laboratory manager: Lauren Eveland

Our project

The overall objective of our research is to obtain and characterize new strains of *Streptomyces* from soil samples. to give you the opportunity to discover new strains you will obtain isolates "from scratch," that is, directly from a soil sample. You will conduct an initial characterization of each isolate, including sequencing part of the 16S rRNA gene, attempting an initial identification using the BLAST search tool, and conducting some observations on phenotype.

We will also provide you with partially characterized isolates from a previous semester. These will be cultures that appear to produce biologically active compounds, cultures that may represent a relatively uncommon group, cultures revealing partial 16S sequences that did not match a known strain in the database, and/or other characteristics of interest. We will provide

you with all of the information collected on these unknowns to date so that you may follow up with further characterization of the genome and phenotype. If you are able to obtain a carefully constructed and complete 16S rRNA sequence, then we may be able to submit the information to the NIH database.

Preparing for the course

Please plan to apply the skills and best practices that you learned in BIOS (BIOC) 211, Intermediate Experimental Biosciences (course prerequisite) and previous experience in research and/or advanced laboratory courses.

Prior to the first day

- Contact your team members and arrange for meetings outside of class time. Tentative team assignments will be announced at least a week before the first day of classes.
- Assign two team members responsibility for collecting a soil sample (preferably from off-campus), to be delivered to the laboratory classroom during the first week of lab.
- See that you have the required materials, listed below.

Required materials

You will need eye protection for this course, consisting of a pair of lab goggles or laboratory safety glasses with side protection. A lab coat will be provided. There is no textbook. You will need to make entries into a laboratory notebook, either a new one or a partly filled notebook from another course. The notebook must be bound, not loose leaf, and designed for use as a laboratory notebook. See the document BIOS 318 notebook guidelines, in the Lab documents folder, for details. We will have you leave the original notebook in the laboratory at the end of the course. You are welcome to make duplicate copies for your own use and you may pick up the original notebook later, after the grading is complete.

Assignments

Your individual and team's laboratory performance will be evaluated in part on the basis of these assignments and in part on observations made by the instructors, laboratory manager, and TAs.

- Laboratory notebook, to be left in the lab at the end of the course
- Three "sequencing assignments" on evaluating chromatograms, obtaining sequencing data, assembling a partial 16S rRNA sequence, and using the NCBI search tools to identify probable species candidates
- A single write-up of your individual and team's efforts to isolate and identify *Streptomyces* species from your soil samples
- An evaluation of your team's effectiveness

Course timetable

Classes will begin with Zoom sessions during the first two weeks, with laboratory work to begin the third week (Jan 24/25). Details are in the file "Detailed activities for S22.pdf." Please peruse the entire document so that you are familiar with our plans for the semester and review the relevant details prior to each class day.

Mon/Tues Jan 10/11

Preparation: Read over the document "Detailed activities for S22.pdf." Before starting any lab work you must review the documents "General lab safety" and "BIOC 318 safety."

In class: We will conduct introductions (students, instructors, lab manager, and TAs) and introduce the course and the project.

Weds/Thurs Jan 12/13

There will be a presentation on genomic DNA isolation, PCR work, and collecting sequencing data.

Weds/Thurs Jan 19/20

We will conduct a presentation on assembling a partial 16S rRNA sequence from chromatogram data.

Mon/Tues Jan 24/25

You will process soil samples "for real" today and begin learning to work with *Streptomyces*

Weds/Thurs Jan 26/27

Today you will begin isolating probable *Streptomyces* bacteria from the spread plates that you prepared two days earlier and learn to view living cultures in a compound microscope.

Mon/Tues Jan 31/Feb 1

Today you will continue to obtain isolates from your spread plates; we will conduct Gram stains and view them in a compound microscope.

Weds/Thurs Feb 2/3

We will conduct a presentation on using the BLAST tool to search the NCBI database of 16S rRNA sequences and on using Bergey's manual to find species descriptions.

Mon/Tues Feb 7/8

We will discuss strategies for further characterization of isolates, including efforts to extend the 16S rRNA sequence and to evaluate isolates for antimicrobial activity.

From here on out, teams will conduct DNA work and assays for antimicrobial activity with the aid of the instructors and TAs.

Additional information and policies

Absences. For full credit for your efforts you are expected to participate in all scheduled meetings unless otherwise advised or due to circumstances beyond your control.

Lab safety. Of course everyone knows the rules and how important it is to stick with them. If you catch one of us violating safety rules then let an instructor know. You may earn bonus credit!

Late assignments. For full credit and for the full benefit of instructor feedack, submit all assignments by the due date and time.

Honor code policy. As with all biosciences courses, the Rice University Honor Code applies to all personal conduct and assignments. All work submitted under your name must be your own work, not copied from another source nor completed with unauthorized assistance. Sources of information must be properly cited. Compliance with the honor code includes providing accurate information when requested, such as honest evaluations of yourself and your teammates.

Students with disabilities. If you have a documented disability that may affect academic performance, you should: 1) make sure this documentation is on file with Disability Resource Center (Allen Center, Room 111 / adarice@rice.edu / x5841) to determine the accommodations you need; and 2) meet with me to discuss your accommodation needs. Of course if you suspect that you have a disability, please contact the Disability Resource Center for an evaluation.